Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 6070–6074, July 1992
Immunology

β_2-Microglobulin$^-$, CD8$^+$ T-cell-deficient mice survive inoculation with high doses of vaccinia virus and exhibit altered IgG responses

(viral immunity/class I expression/transgenic mice)

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Contributed by Oliver Smithies, March 12, 1992

ABSTRACT **Transgenic mice lacking an intact β_2-microglobulin (β_2m) gene fail to express major histocompatibility complex (MHC) class I proteins on the cell surface and, as a result, are virtually devoid of CD4$^-$ CD8$^+$ lymphocytes. These animals provide a unique model system for directly assessing the role of CD8$^+$ lymphocytes in the modulation of viral infection *in vivo*. β_2m$^-$ CD8$^-$ mice and their normal littermates were inoculated at the base of the tail with the WR strain of vaccinia virus and monitored for serum antibody and lesion formation. Both groups developed similar lesions in response to a broad virus dose range, and all animals had completely recovered by day 28 after inoculation. Isotype-specific immunoglobulin levels were determined for each animal on day 7 and day 14 after primary inoculation, and again 7 days after a virus challenge. The virus-specific IgG1, IgG2a, and IgG2b levels were significantly different in the β_2m$^-$/− group (20-, 9-, and 30-fold lower, respectively, on day 7 after challenge) compared with the β_2m$^+$/− group. Virus-specific serum IgM levels for both groups remained similar throughout the experiment. In a separate experiment, β_2m$^-$/− mice were immunized with a nonviral antigen, 2,4,6-trinitrophenyl-conjugated keyhole limpet hemocyanin, and both total and antigen-specific isotype-specific immunoglobulin titers were determined. Total IgG1, IgG2a, IgG2b, and IgG3 tended to be lower overall in the β_2m$^-$/− mice compared with β_2m$^+$/− littermates. In contrast, total and antigen-specific IgE titers were similar in the two groups. These data indicate that CD8$^+$ lymphocytes are not required to clear high doses of vaccinia virus, and they suggest that β_2m$^-$/− mice are less efficient at antigen-specific IgG production than their β_2m$^+$/− littermates.**

Cytolytic T lymphocytes (CTL) have been reported to play an important role in host modulation of viral infections. Virus-specific CD4$^-$ CD8$^+$ CTL can be detected prior to neutralizing antibody, as early as 4 days after infection with viruses such as ectromelia or influenza (1, 2). It is thought that CTL are able to eliminate virally infected host cells prior to the release of progeny virus particles, resulting in effective limitation or early clearance of the viral infection (3, 4). CD8$^+$ CTL recognize infected cells through the T-cell receptor, which binds to virus-specific peptides displayed on the cell surface in conjunction with major histocompatibility complex (MHC) class I proteins (5, 6). Class I MHC molecules on the cell surface are noncovalently associated with a small β_2-microglobulin (β_2m) peptide (7, 8). In the absence of expression of β_2m, the transport of class I MHC molecules is arrested in the endoplasmic reticulum and the MHC molecules are not available for cell surface expression and antigen presentation (9, 10). Recently, stable murine lines carrying a disrupted β_2m gene have been developed (11, 12). These mice appear normal; however, they lack detectable MHC class I molecules on their cell surfaces, and they are almost completely devoid of CD4$^-$ CD8$^+$ lymphocytes. These mice thus provide an excellent opportunity to examine the role of CD8$^+$ T cells and class I MHC-mediated antigen presentation in the modulation of virus infection.

Vaccinia virus (VV) is a prototypic member of the poxvirus family. These viruses typically elicit a strong cell-mediated immune response in the infected host. The WR strain of VV is a neurovirulent strain that was isolated by repeated passage in suckling mouse brain (13). The LD_{50} varies with the route of administration but is greater than 10^9 plaque-forming units (pfu) when given intradermally (i.d.) in normal mice (14). Lesions, the size of which can vary with the strain of mouse immunized, may appear at the site of inoculation. Nude mice inoculated with as few as 10 pfu of VV develop generalized vaccinia and typically die within 28 days (M.K.S., unpublished observations).

We have examined the role of CD8$^+$ cells in the modulation of VV infection by using β_2m$^-$/− CD8$^-$ transgenic mice. β_2m$^-$/− mice and their normal littermates were examined for lesion formation in response to a broad virus dose range and were serologically monitored for virus-specific isotype-specific antibody production. In this paper, we report that (*i*) β_2m$^-$/− mice survived infection with up to 5×10^8 pfu of VV; (*ii*) the course of infection in β_2m$^-$/− mice appeared indistinguishable from that seen in β_2m$^+$/− littermates; and (*iii*) β_2m$^-$/− mice produced less virus-specific IgG antibodies after infection than did their β_2m$^+$/− littermates, although both groups produced similar levels of virus-specific IgM.

We compared the antibody response of these mice to the response to a nonviral soluble antigen, 2,4,6-trinitrophenyl-conjugated keyhole limpet hemocyanin (TNP-KLH). This antigen typically elicits strong secondary IgG1 and IgE responses and provides an alternative method for examining an antigen-specific immunoglobulin response (15). These experiments showed that β_2m$^-$/− mice produced somewhat less TNP-KLH-specific IgG1 than did their normal littermates, but both groups produced equivalent levels of TNP-KLH-specific IgE.

MATERIALS AND METHODS

Animals and Antigens. β_2m-deficient mice were derived by the introduction of E1422A embryonic stem cells carrying a disrupted β_2m gene into C57BL/6 mice (11). Chimeric off-

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Abbreviations: CTL, cytolytic T lymphocytes; MHC, major histocompatibility complex; β_2m, β_2-microglobulin; VV, vaccinia virus; pfu, plaque-forming units; TNP-KLH, 2,4,6-trinitrophenyl-conjugated keyhole limpet hemocyanin; mAb, monoclonal antibody; sIgM, surface IgM.

spring were selected on the basis of coat color and then backcrossed to C57BL/6 mice. The resulting heterozygous offspring were mated to give the F_2 animals used in these experiments. Southern blot analysis of tail DNA was performed on individual animals to determine genotype. The WR strain of VV was purchased from the American Type Culture Collection and plaque purified as described (16). TNP-KLH was precipitated with alum under pyrogen-free conditions. Intraperitoneal injections were performed with 1 μg of TNP-KLH in a final volume of 200 μl.

ELISAs. VV-specific ELISAs were performed essentially as described (17), using horseradish peroxidase-conjugated isotype-specific antibodies (Southern Biotechnology Associates, Birmingham, AL). Antiviral antibody titers are reported as half-maximal units per ml and are derived as follows: Immune "standard" serum from a single heterozygous $\beta_2m^+/-$ mouse was used to determine a maximum possible OD value for each isotype. A titration of this standard serum was performed for the appropriate isotype-specific reagent on each plate to control for plate-to-plate variation. Half-maximal values are arbitrary units equivalent to the amount of antibody present in the dilution of test serum required to give an OD reading equal to one-half the maximal OD value for the standard serum. All titrations were performed in duplicate and curves were generated from the average OD value per point. Half-maximal values were calculated by using the BIOASSAY program. Full documentation of the BIOASSAY program set can be found in *Bioassay Analysis Program Documentation Protocol* (Immunex: August 1990). Calculations consist of eliminating unusable data points, calculating fractional percentages of assay maxima, logit transforming data, performing least-squares line fits, and plotting as dilution of serum vs. OD. The lower limit of detectability in these assays was 2.5 units/ml. Serum samples with a half-maximal value less than 2.5 units/ml were assigned a value of zero for purposes of statistical analysis. Student's *t* tests were performed by using STATWORKS version 1.2, 1985 (Cricket Software, Philadelphia). Total isotype levels were determined by an isotype-specific sandwich ELISA as described previously (18). Primary and secondary anti-TNP IgG1 and IgE levels were determined by a modified sandwich ELISA method. Plates were coated with anti-murine IgG1 or IgE antibodies, blocked with nonfat dry milk, and incubated with test serum samples. Bovine gamma globulin was 2,4,6-trinitrophenylated and biotinylated as described (19, 20) and added to each well, followed by horseradish peroxidase-conjugated streptavidin. Wells were developed with peroxidase substrate and analyzed as described above. Dinitrophenyl-specific myeloma antibodies [TIB 191 (IgG1) and A3B1 (IgE)] were used as standards to determine antigen-specific IgG1 and IgE levels.

Immunofluorescent Staining and Flow Cytometry. The following monoclonal antibodies (mAbs) were used in these studies: 2A3.6B2 mAb, which specifically recognizes Ly5/B220 antigen on B-lineage cells (21); 25-9-17 mAb, which recognizes I-A^b (22); 28-8-8 mAb, which recognizes K^b, D^b (22); 2.4G2 mAb, which recognizes the Fcγ receptor (23); 53-12.7 mAb, which recognizes murine CD8 (24); and GK1.5 mAb, which recognizes murine CD4 (25). For assessment of the expression of B220 and surface IgM (sIgM) on lymph node and spleen cells, cells were incubated with phycoerythrin (PE)-conjugated goat anti-mouse IgM (Southern Biotechnology Associates) and fluorescein isothiocyanate (FITC)-conjugated anti-B220. To assess the expression of CD4 and CD8, cells were preincubated with 2.4G2 mAb, washed, and incubated with PE-conjugated anti-CD4 and FITC-conjugated anti-CD8 (Becton Dickinson). To assess the expression of class I or II MHC antigens, cells were incubated with either 28-8-8 mAb or 25-9-17 mAb, washed, and incubated with FITC-conjugated F(ab')$_2$ fragment of goat antibody specific for the Fc fragment of mouse IgG (Cappel Laboratories). Analysis was performed on a FACScan flow cytometer (Becton Dickinson). Background staining was assessed by incubating the cells with isotype-matched antibodies of irrelevant specificity followed by the second step antibody or by staining with FITC- or PE-conjugated mouse IgG1 (Becton Dickinson). For two-color analysis, cells stained with each antibody alone were first analyzed to determine the degree of spectral overlap.

RESULTS

Lesion Formation After Inoculation with VV. Homozygous $\beta_2m^-/-$ mice and their normal heterozygous $\beta_2m^+/-$ littermates were inoculated i.d. at the base of the tail with 5×10^2, 5×10^5, or 5×10^8 pfu of VV. Three mice of each genotype were used for each dose group. Animals were weighed twice weekly and monitored every other day for lesion formation. Lesions were scored on a 1+ to 4+ scale, with 1+ including lesion diameters of up to 2 mm, erythema, or traces of vesicle formation, and 4+ including lesions greater than 1 cm, which were fully vesicular and weeping.

At the site of inoculation all animals formed lesions, the size and severity of which correlated with the dose of virus administered. Mice in the low-dose group developed detectable lesions between days 10 and 12, which peaked by day 14 and were completely healed by day 28. Lesions typically ranged from 1+ to 2+ on day 14 with no difference apparent between the animals lacking $CD8^+$ lymphocytes and their normal littermates. Animals receiving the midrange dose typically developed lesions by day 7, which peaked from day 12 to day 14 and were completely healed by day 28. Lesions ranged from 2+ to 3+ on day 14, again with no difference between the two genotypes at the level of either lesion size or progression. Animals that were given the highest dose (5×10^8 pfu) began developing lesions by day 4 or 5, and by day 14 all animals in both $\beta_2m^-/-$ and $\beta_2m^+/-$ groups had 4+ lesions, some of which had spread to cover most of the tail. All lesions in both groups had completely healed by day 28, although these animals sustained permanent scar tissue on the tail. Interestingly, animals of either genotype in all dose groups experienced no spread of viral lesions to parts of the body other than the tail. No lesions were detected even at peak times on the feet, face, eyes, or oral cavity. At the onset of the study, animals ranged in weight between 20 and 30.1 g. Over the 28-day course of the experiment, all animals experienced weight gains consistent with normal growth in age-matched uninfected mice.

Serum samples were collected on day 0 prior to inoculation, and again on day 7 and day 14. An ELISA was used to determine virus-specific IgM and IgG titers for each animal. All animals were seronegative for VV antibodies at the onset and all developed detectable virus-specific antibodies by day 7. As expected, the levels of IgM and IgG correlated with the amount of virus administered (data not shown). However, it appeared that for each dose of virus administered, the IgG response was less vigorous in the $\beta_2m^-/-$ $CD8^-$ mice than in their littermates. To document this finding more carefully, the experiment was repeated on a broader scale.

Antibody Response of $\beta_2m^-/-$ and $\beta_2m^+/-$ Mice to VV. Ten heterozygous $\beta_2m^+/-$ and eight homozygous $\beta_2m^-/-$ mice were administered 5×10^4 pfu i.d. at the base of the tail. On the basis of the previous study, this dose of virus could be expected to give a vigorous immune response without severe lesion formation. On day 21 after inoculation, animals were challenged intraperitoneally (i.p.) with 10^8 pfu of VV. Serum samples were taken on day 0 prior to inoculation, then on day 7, day 14, and day 28.

After primary inoculation, both $\beta_2m^-/-$ and $\beta_2m^+/-$ mice developed lesions ranging from 1+ to 2+ in size, and all

Table 1. Isotype-specific serum antibody response to VV in $\beta_2m^-/-$ $CD8^-$ mice and their normal littermates

	Anti-VV titer											
	Day 7	Day 14					Day 28					
Mice	IgM	IgM	IgG	IgG1	IgG2a	IgG2b	IgM	IgG	IgG1	IgG2a	IgG2b	IgG3
$\beta_2m^+/-$	3	425	1592	235	584	676	184	2206	1249	1817	474	37
$\beta_2m^-/-$	3	762	301	14	175	159	242	423	59	197	16	<2.5
P	0.911	0.237	<0.001	<0.001	0.232	0.003	0.578	<0.001	<0.001	0.042	0.001	<0.001

Ten heterozygous $\beta_2m^+/-$ mice and eight homozygous $\beta_2m^-/-$ mice were inoculated on day 0 as described in the text. On day 21, animals were boosted with 10^8 pfu of VV i.p. Serum samples were collected on the indicated days and half-maximal ELISA values were determined. The lower limit of detectability in these assays was 2.5 units/ml. The values listed represent geometric means of the half-maximal value for each isotype within a genotypic group. *P* values for homozygous vs. heterozygous mice were determined by using Student's *t* test on the log-transformed data; *P* values <0.05 are considered significant.

lesions resolved by day 28. No morbidity or generalized lesions were observed in any mice after the i.p. challenge.

Virus-specific isotype-specific ELISA was performed with serum obtained from individual animals. The results of these assays are summarized in Table 1, where the values listed represent the geometric mean of the half-maximal serum antibody titers (see *Materials and Methods*) for a given isotype within a genotypic group.

Virus-specific IgM titers were not significantly different between the $\beta_2m^-/-$ and $\beta_2m^+/-$ mice at any time of testing during the course of the experiment. Virus-specific serum IgM levels were increased in both groups on day 14 compared with day 7, but they did not significantly increase after the i.p. challenge. In contrast, virus-specific IgG titers were greater in $\beta_2m^+/-$ normal mice on day 14 than in $\beta_2m^-/-$ transgenic mice. After the challenge (day 28), all IgG titers increased in all animals, and the disparity between the IgG isotype titer in $\beta_2m^-/-$ and $\beta_2m^+/-$ mice was even more pronounced. At this time, the IgG1, IgG2a, and IgG2b titers differed by 20-, 9-, and 30-fold, respectively. The magnitude of difference in IgG3 levels is difficult to calculate accurately, as $\beta_2m^-/-$ frequently had nondetectable levels of this isotype. All $\beta_2m^+/-$ mice, however, did respond to inoculation by producing detectable levels of IgG3.

Table 1 also lists Student's *t* test *P* values comparing $\beta_2m^+/-$ and $\beta_2m^-/-$ titers for each isotype at day 14 and day 28. At day 28, all IgG isotype levels were significantly different in the $\beta_2m^-/-$ and $\beta_2m^+/-$ groups at the 0.05 level.

Scatter plots depicting the serologic data for individual animals on day 28 are shown in Fig. 1. The IgG titers within the $\beta_2m^-/-$ group tended to be more tightly clustered than in the $\beta_2m^+/-$ group. This was also seen in the titers determined on day 14 (data not shown).

Humoral Immune Response to TNP-KLH. The results described above suggested that expression of class I molecules may be involved in the development of strong anti-viral antibody responses. We sought to determine whether this requirement extends to other antigens by immunizing mice with a nonviral antigen (TNP-KLH) and measuring the antigen-specific isotype levels in serum. TNP-KLH is a hapten-carrier conjugate that has been shown to elicit strong T-cell-dependent antibody responses in mice, particularly of the IgE and IgG1 isotypes (15). Mice were immunized with TNP-KLH on day 0 and boosted on day 21, and primary (day 9) and secondary (day 26) anti-TNP responses were measured by ELISA analysis of sera. Fig. 2 *A* and *B* compares the total and anti-TNP-specific IgE levels in sera from 9 $\beta_2m^-/-$ and 10 $\beta_2m^+/-$ mice. Mice in both groups mounted equivalent secondary anti-TNP IgE responses. Comparison of total and anti-TNP IgE levels indicates that the majority of the total IgE present was antigen-specific, which is typical of anti-TNP IgE responses in normal mice. It is noteworthy that the ranges of secondary anti-TNP IgE responses for $\beta_2m^-/-$ (0.88–13.37 μg/ml) and $\beta_2m^+/-$ (0.033–20.57 μg/ml) mice were quite broad. The animals used in this study represent a diverse genetic background, being an F_2 generation outbred from 129/Ola × C57BL/6 (see *Materials and Methods*). This diversity may account for the wide range of antigen-specific IgE responses in the mice. Nevertheless, the results strongly suggest that class I antigen expression neither is required nor

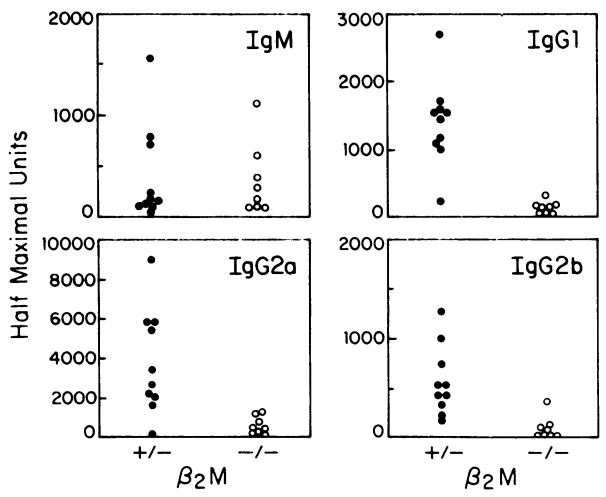

FIG. 1. Scatter plots depicting VV-specific serum antibody responses from individual $\beta_2m^-/-$ and $\beta_2m^+/-$ mice on day 28. Mice were inoculated as described in the text. Isotype-specific ELISA was performed on serum samples taken 7 days after challenge. The minimum level of detectability in this assay was 2.5 units/ml.

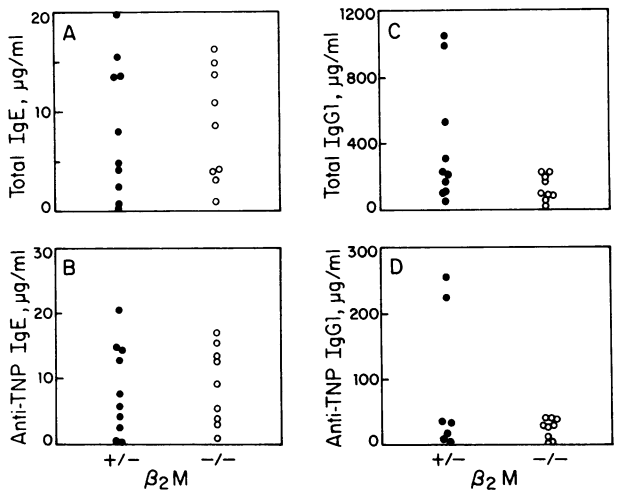

FIG. 2. Serum anti-TNP and total IgE and IgG1 secondary responses in mice immunized with TNP-KLH. Day 26 sera (5 days after secondary immunization) were assayed for total (*A* and *C*) or TNP-specific (*B* and *D*) immunoglobulin levels by isotype-specific sandwich ELISAs. Each point represents IgE (*A* and *B*) or IgG1 (*C* and *D*) in sera from individual $\beta_2m^-/-$ (○) or $\beta_2m^+/-$ (●) mice.

Table 2. Total serum isotype levels in $\beta_2m^+/-$ and $\beta_2m^-/-$ mice before and after immunization and challenge with TNP-KLH

Sera	Mice	Isotype concentration, μg/ml					
		IgE	IgG1	IgG2a	IgG2b	IgG3	IgM
Preimmune	$\beta_2m^-/-$	<0.01	11.89 ×/÷ 1.31	4.08 ×/÷ 1.33	18.75 ×/÷ 1.30	15.60 ×/÷ 1.37	1199 ×/÷ 1.15
	$\beta_2m^+/-$	<0.01	92.33 ×/÷ 1.24	13.40 ×/÷ 1.34	58.28 ×/÷ 1.32	83.47 ×/÷ 1.25	776 ×/÷ 1.23
Primary	$\beta_2m^-/-$	0.04 ×/÷ 1.56	21.97 ×/÷ 1.31	5.94 ×/÷ 1.42	29.41 ×/÷ 1.25	29.54 ×/÷ 1.43	1662 ×/÷ 1.12
	$\beta_2m^+/-$	0.02 ×/÷ 1.40	116.30 ×/÷ 1.21	18.40 ×/÷ 1.36	88.76 ×/÷ 1.36	139.66 ×/÷ 1.21	1202 ×/÷ 1.19
Secondary	$\beta_2m^-/-$	6.14 ×/÷ 1.38	106.57 ×/÷ 1.28	5.69 ×/÷ 1.38	39.93 ×/÷ 1.40	40.63 ×/÷ 1.34	1948 ×/÷ 1.16
	$\beta_2m^+/-$	3.58 ×/÷ 1.87	239.78 ×/÷ 1.36	19.70 ×/÷ 1.31	85.99 ×/÷ 1.38	176.67 ×/÷ 1.28	1542 ×/÷ 1.17

Values are reported as geometric means ×/÷ SEM of total isotype in sera from $\beta_2m^+/-$ ($n = 10$) and $\beta_2m^-/-$ ($n = 9$) mice. Preimmune, primary, and secondary bleedings were performed on day 0, day 9, and day 26, respectively, after primary immunization with TNP-KLH.

has a major influence on the generation of an antigen-specific IgE response.

Serum IgG1 was also measured in both groups of mice. The results in Fig. 2*C* show that total serum IgG1 levels after challenge with TNP-KLH tended to be lower in $\beta_2m^-/-$ mice than in $\beta_2m^+/-$ mice. The anti-TNP IgG1 results (Fig. 2*D*) are less conclusive due to the presence of two high responders in the $\beta_2m^+/-$ group. If these two outlier values are disregarded, the IgG1 anti-TNP levels for the two groups are similar. These findings suggest that class I antigen-deficient mice are capable of mounting a normal TNP-specific IgG1 response, although interpretation of these results is hampered by the wide range of values that were observed for individual mice.

The levels of total serum isotypes in preimmune, primary, and secondary sera are listed in Table 2. The preimmune sera results indicate that the $\beta_2m^-/-$ mice had lower total IgG1, IgG2a, IgG2b, and IgG3 than littermate controls. Moreover, the isotype levels in $\beta_2m^-/-$ mice remain lower than those in $\beta_2m^+/-$ mice throughout the course of the immunization period. Interestingly, examination of the difference in IgG1 levels within the two groups indicates that the $\beta_2m^-/-$ animals exhibit a greater than 9-fold increase in secondary IgG1 levels compared with preimmunization levels, while $\beta_2m^+/-$ showed a less than 3-fold increase. In contrast to the IgG results, there did not appear to be a difference between groups in the ability to produce IgE or IgM. Thus, $\beta_2m^-/-$ animals can mount a humoral response to soluble antigen, but they clearly exhibit a deficiency in levels of IgG subclass production.

Phenotypic Analysis of Lymphoid Tissue. Spleen and mesenteric lymph nodes from normal C56BL/6, $\beta_2m^+/-$, and $\beta_2m^-/-$ mice were assessed for the numbers of cells expressing CD4, CD8, and B220/sIgM by immunofluorescent staining and flow cytometric analysis. As seen in Table 3, $\beta_2m^-/-$ mice had a significantly greater total number of spleen cells than either the $\beta_2m^+/-$ mice or C57BL/6 mice. The increased splenic cellularity was due to greater numbers of $CD4^+$ cells, $sIgM^+$ cells, and cells negative for both of these markers (null cells). These data indicate that the decrease of IgG in the $\beta_2m^-/-$ homozygotes is not a result of a diminished number of $B220^+/sIgM^+$ precursor cells. Interestingly, analysis of the mesenteric lymph nodes from these same mice revealed no difference in total cellularity or in the numbers of $CD4^+$, $sIgM^+$, or null cells between the groups (data not shown).

DISCUSSION

The cell-mediated component of the immune system is considered to be of equal if not greater importance than humoral immunity in the modulation of pathogenesis of many infectious diseases. Studies performed with virus-infected mice have led to the widely accepted notion that $CD8^+$ CTL are the primary mediators of immunity against viruses such as poxviruses and influenza. Virus-specific $CD8^+$ CTL are readily isolated from animals infected with these viruses, and *in vitro* studies have clearly identified viral proteins that are recognized by CTL in association with class I MHC molecules on the surface of infected cells (refs. 26 and 27; for a review, see ref. 28). Adoptive transfer experiments, in which lethal infections can be abrogated by infusion of CTL in the absence of neutralizing antibody, provide even more compelling evidence for a critical role of CTL in the clearance of poxviruses (29, 30).

In the case of VV, the evidence for the importance of cell-mediated immunity in limitation and recovery from disease comes primarily from data gathered during the years of widespread immunization against smallpox in humans. Patients with known cell-mediated immune dysfunction typically developed severe generalized rashes after immunization (31), which was characteristic of progressive vaccinia (vaccinia necrosum). In mice, the evidence for the importance of CTL in immunity is indirect because VV is not typically lethal unless administered intracranially. To test directly the role of $CD8^+$ CTL in the clearance of VV infection, we investigated the progression of VV infection in mice lacking $CD8^+$ T cells.

$\beta_2m^-/-$ mice and their normal littermates were inoculated with various doses of VV. Animals of both genotypes developed similar lesions, the severity of which were dose-dependent. All animals survived inoculation, even at doses exceeding 10^8 pfu, and experienced little if any outward signs of viremia or illness. These results demonstrate directly that $CD8^+$ CTL are not required for the clearance of VV in these mice. Although the $CD8^+$ cells are likely to play an important role in the modulation of viral infections in a normal animal, it is clear that the immune system provides sufficient overlap to protect $\beta_2m^-/-$ from VV infection under these conditions.

Table 3. Phenotypic analysis of splenic lymphocyte populations

Mice	Cells × 10^{-6}				
	Total cellularity	$CD4^+$ $CD8^-$	$CD4^-$ $CD8^+$	$B220^+/sIgM^+$	Null
C57BL/6	133.8 ± 8.2	17.3 ± 2.4	12.8 ± 1.8	81.9 ± 6.7	20.8 ± 2.6
$\beta_2m^+/-$	152.2 ± 13.1	15.1 ± 1.5	11.1 ± 2.6	103.8 ± 11.3	21.7 ± 1.1
$\beta_2m^-/-$	213.7 ± 15.4	24.2 ± 3.4	0.8 ± 0.8	138.8 ± 15.1	49.6 ± 9.5

The data are the arithmetic mean ± SEM of 5 individually assayed mice. The numbers of $CD4^+$, $CD8^+$, and $sIgM^+$ cells are the product of the percent positively staining cells and the total cellularity of the spleen. The numbers of null cells are the sum of the numbers of $CD4^+$, $CD8^+$, $sIgM^+$ cells subtracted from the total cellularity.

The exact nature of the effector mechanisms is not known. One explanation might be that other cytolytic cells such as $CD4^+$ CTL and/or natural killer (NK) cells play a prominent role in viral clearance in the absence of $CD8^+$ CTL. Indeed, phenotypic analysis of splenic lymphoid cells of $\beta_2m^-/-$ mice (Table 3) showed increased $CD4^+$ and null cell subsets compared with their littermate controls. Consistent with this hypothesis, Karupiah *et al.* (32) have shown that nude mice infected with a recombinant VV expressing interleukin 2 survive infection and exhibit elevated NK cell activity. NK activity has also been shown to be important in the resistance of mice to a variety of herpesvirus infections (33–36) and perhaps is critical to resistance to herpesvirus infection in humans (37).

An unexpected finding regarding the ability of $\beta_2m^-/-$ mice to mount an antibody response to VV emerged from these studies. Virus-specific antibody titers were examined by ELISA after inoculation and virus challenge. The levels of virus-specific IgG were significantly lower in the $\beta_2m^-/-$ group than in their normal littermates, while serum IgM levels were similar. To investigate whether this difference was limited to a response to viral antigens, a similar experiment was performed in which $\beta_2m^-/-$ and their normal littermates were immunized with a nonviral hapten carrier, TNP-KLH, which elicits strong IgE and IgG1 responses in normal mice. In this experiment, amounts of antigen-specific IgE in the serum of $\beta_2m^-/-$ and $\beta_2m^+/-$ mice were similar; a large degree of variability in the IgG1 response made results for this subclass difficult to interpret. However, examination of total serum antibody (non-antigen-specific) (Table 2) indicated that total IgM and IgE levels were similar in the two groups but that total IgG levels were lower in $\beta_2m^-/-$ mice than in $\beta_2m^+/-$ controls. This was true for both preimmune sera and sera taken after immunization or challenge and applied to all IgG isotypes. When these data are considered together with data on virus-specific IgG in $\beta_2m^-/-$ mice, it appears that mice lacking β_2m are less efficient in the generation of IgG antibody than their normal littermates and that class I antigen expression is not required for the generation of an antigen-specific IgE response.

The mechanism responsible for diminished production of certain IgG isotypes in $\beta_2m^-/-$ mice is unclear. The numbers of B cells as defined by B220 and sIgM were comparable between $\beta_2m^-/-$ mice and their normal littermates. Given the role of cytokines in class switching, it is tempting to speculate that a cytokine normally produced by $CD8^+$ cells may be response limiting in mice lacking these cells. For example, this isotype distribution pattern might be predicted if γ interferon were limiting in the $\beta_2m^-/-$ mice. This cytokine is known to be secreted by $CD8^+$ cells as well as $CD4^+$ cells. It is of interest that our serum antibody data differ from those reported by Fung-Leung *et al.* (38) for vesicular stomatitis virus (VSV) infection of transgenic mice devoid of $CD8^+$ T cells owing to a disrupted Lyt-2 gene. In that study, VSV-neutralizing IgM and IgG antibodies were measured and found to be essentially the same between the $CD8^-$ transgenic mice and wild-type mice. As different antigens were used to elicit responses in these two studies, and because total serum antibody titers in these mice prior to immunization were not reported, direct comparison of the data is not possible. The possibility is raised that the absence of β_2m limits the antibody response by an undefined mechanism not related to the absence of $CD8^+$ cells. Further studies will be necessary to clarify this issue.

The authors thank Melvin Steggs for assistance in the rapid dissemination of information regarding these studies.

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